REMARKS

New claims 16-28 are added by this amendment. These claims are supported by the original disclosure as detailed below.

Claim	Citations to paragraphs supporting the new claims	Supporting Language
16	Page 10, line 30; page 11, line 11; page 15, line 1; page 37, line 9 to page 38, line 34	As discussed above, in one embodiment, the above-described object of the present invention have been met by a peptide antagonist of zonulin comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, and SEQ ID NO:35, wherein said peptide antagonist binds to ZOT receptor, yet does not physiologically modulate the opening of mammalian tight junctions. *** The size of the peptide antagonist is not critical to the present invention. Generally, the size of the peptide antagonist will range from 8 to 110, amino acids, preferably from 8 to 40 amino acids, more preferably will be 8 amino acids. *** The peptide antagonists can be used as to inhibit breakdown of the blood brain barrier. Thus, the peptide antagonists of the present invention are useful, e.g., in the treatment of conditions associated with breakdown of the blood brain barrier. Examples of such conditions include osmotic injuries, e.g., cerebral ischemia, stroke or cerebral edema; hypertension; carbon dioxide; convulsive seizure; chemical toxins; uremia (renal insufficiency); meningitis, encephalitis, encephalomielitis, e.g., infective (viral (SRV, HIV, etc.), or bacterial (TB, H. influenzae, meningococcus, etc.) or allergic; tumors; traumatic brain injuries; radiation brain injury; immaturity and kernicterus; demyelinating diseases, e.g., multiple sclerosis or Guillian-Barre syndrome. *** Given that ZOT, human intestinal zonulin (zonulin.sub.i) and human heart zonulin (zonulin.sub.h) all act on intestinal (Fasano et al, Gastroenterology, 112:839 (1997); Fasano et al,

J. Clin. Invest., 96:710 (1995); and FIGS. 1-5) and endothelial tj and that all three have a similar regional effect (Fasano et al (1997), supra; and FIGS. 1-5) that coincides with the ZOT receptor distribution within the intestine (Fasano et al (1997), supra; and Fasano et al (1995), supra) , it was postulated in the present invention that these three molecules interact with the same receptor binding site. A comparison of the primary amino acid structure of ZOT and the human zonulins was thus carried out to provide insights as to the absolute structural requirements of the receptor-ligand interaction involved in the regulation of intestinal tj. The analysis of the N-termini of these molecules revealed the following common motif (amino acid residues 8-15 boxed in FIG. 7): non-polar (Gly for intestine, Val for brain), variable, non-polar, variable, non-polar, polar, variable, polar (Gly). Gly in position 8, Val in position 12 and Gln in position 13, all are highly conserved in ZOT, zonulin.sub.i and zonulin.sub.h (see FIG. 7), which is believed to be critical for receptor binding function within the intestine. To verify the same, the synthetic octapeptide Gly Gly Val Leu Val Gln Pro Gly (SEQ ID NO:15) (named FZI/0, and corresponding to amino acid residues 8-15 of human fetal zonulin.sub.i) was chemically synthesized.

Next, rabbit ileum mounted in Ussing chambers as described above, were exposed to 100 .mu.g of FZI/0 (SEQ ID NO:15), 100 .mu.g ofFZI/1 (SEQ ID NO:34), 1.0 .mu.g of 6xHis-ZOT (obtained as described in Example 1), 1.0 .mu.g of zonulini (obtained as described in Example 3), or 1.0 .mu.g of zonulin.sub.h (obtained as described in Example 3) alone; or pre-exposed for 20 min to 100 .mu.g of FZI/0 or FZI/1, at which time 1.0 .mu.g of 6xHis-ZOT, 1.0 .mu.g of zonulin.sub.i, or 1.0 .mu.g of zonulin.sub.h, was added. .DELTA.Rt was then calculated as described above. The results are shown in FIG. 8.

As shown in FIG. 8, FZI/0 did not induce any significant change in Rt (0.5% as compared to the negative control) (see closed bar). On the contrary, pre-treatment for 20 min with FZI/0 decreased the effect of ZOT, zonulin.sub.i, and zonulin.sub.h on Rt by 75%, 97%, and 100%, respectively (see open bar). Also as shown in FIG. 8, this inhibitory effect was completely ablated when a second synthetic peptide (FZI/1) was chemically synthesized by changing the Gly in position 8, the Val in position 12, and the Gln in position 13 (as referred to zonulin.sub.i) with the correspondent amino acid residues of zonulin.sub.b (Val, Gly, and Arg,

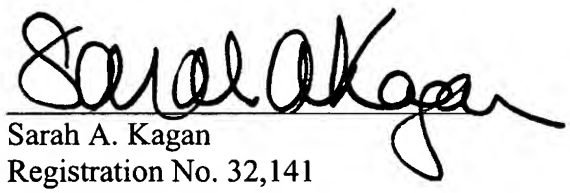
		respectively) was used (see shaded bar). The above results demonstrate that there is a region spanning between residue 8 and 15 of the N-terminal end of ZOT and the zonulin family that is crucial for the binding to the target receptor, and that the amino acid residues in position 8, 12, and 13 determine the tissue specificity of this binding.
17-27	Page 34, line 1	The N-terminal sequence of zonulin purified from adult human brain (SEQ ID NO:29) and fetal human brain (SEQ ID NO:36) was completely different than the N-terminal of zonulin purified from each of heart (SEQ ID NO:28), fetal intestine (SEQ ID NO:30) and adult intestine (SEQ ID NO:31) (see FIGS. 6-7). This difference is believed to explain the tissue-specificity of zonulin in determining the permeability of tissues, such as the intestine, demonstrated above.
28	Page 37, line 9 to page 38, line 34	Given that ZOT, human intestinal zonulin (zonulin.sub.i) and human heart zonulin (zonulin.sub.h) all act on intestinal (Fasano et al, Gastroenterology, 112:839 (1997); Fasano et al, J. Clin. Invest., 96:710 (1995); and FIGS. 1-5) and endothelial tj and that all three have a similar regional effect (Fasano et al (1997), supra; and FIGS. 1-5) that coincides with the ZOT receptor distribution within the intestine (Fasano et al (1997), supra; and Fasano et al (1995), supra) , it was postulated in the present invention that these three molecules interact with the same receptor binding site. A comparison of the primary amino acid structure of ZOT and the human zonulins was thus carried out to provide insights as to the absolute structural requirements of the receptor-ligand interaction involved in the regulation of intestinal tj. The analysis of the N-termini of these molecules revealed the following common motif (amino acid residues 8-15 boxed in FIG. 7): non-polar (Gly for intestine, Val for brain), variable, non-polar, variable, non-polar, polar, variable, polar (Gly). Gly in position 8, Val in position 12 and Gln in position 13, all are highly conserved in ZOT, zonulin.sub.i and zonulin.sub.h (see FIG. 7), which is believed to be critical for receptor binding function within the intestine. To verify the same, the synthetic octapeptide Gly Gly Val Leu Val Gln Pro Gly (SEQ ID NO:15) (named FZI/0, and corresponding to amino acid residues 8-15 of human fetal zonulin.sub.i) was chemically synthesized. Next, rabbit ileum mounted in Ussing chambers as described above, were exposed to 100 .mu.g of FZI/0 (SEQ ID NO:15), 100 .mu.g ofFZI/1 (SEQ ID NO:34), 1.0 .mu.g of 6xHis-ZOT (obtained as described in Example 1), 1.0 .mu.g of zonulini

		(obtained as described in Example 3), or 1.0 .mu.g of zonulin.sub.h (obtained as described in Example 3) alone; or pre-exposed for 20 min to 100 .mu.g of FZI/0 or FZI/1, at which time 1.0 .mu.g of 6xHis-ZOT, 1.0 .mu.g of zonulin.sub.i, or 1.0 .mu.g of zonulin.sub.h, was added. .DELTA.Rt was then calculated as described above. The results are shown in FIG. 8. As shown in FIG. 8, FZI/0 did not induce any significant change in Rt (0.5% as compared to the negative control) (see closed bar). On the contrary, pre-treatment for 20 min with FZI/0 decreased the effect of ZOT, zonulin.sub.i, and zonulin.sub.h on Rt by 75%, 97%, and 100%, respectively (see open bar). Also as shown in FIG. 8, this inhibitory effect was completely ablated when a second synthetic peptide (FZI/1) was chemically synthesized by changing the Gly in position 8, the Val in position 12, and the Gln in position 13 (as referred to zonulin.sub.i) with the correspondent amino acid residues of zonulin.sub.b (Val, Gly, and Arg, respectively) was used (see shaded bar). The above results demonstrate that there is a region spanning between residue 8 and 15 of the N-terminal end of ZOT and the zonulin family that is crucial for the binding to the target receptor, and that the amino acid residues in position 8, 12, and 13 determine the tissue specificity of this binding.

It is respectfully submitted that the new claims add no new matter to the application. Examination of the pending claims is respectfully requested.

Respectfully submitted,

Dated: October 8, 2004

By: 

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